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THE SYSTEMATIC STATUS OF *MYTILUS GALLOPROVINCIALIS* IN WESTERN EUROPE: A REVIEW

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ABSTRACT

The controversy concerning the systematic status of the Mediterranean mussel *Mytilus galloprovincialis* Lmk. dates back to the 1860s. While some authorities regard *M. galloprovincialis* as a distinct species, others consider it a variety of *Mytilus edulis* L. Separation of *M. edulis* and *M. galloprovincialis* has traditionally been based primarily on morphological shell characteristics. The first part of this paper discusses the reliability of these characters in separating mixed samples of the two types of mussel in W Europe. The conclusion is that with few exceptions—e.g. Rock in SW England, where there is some evidence of premating isolating mechanisms—separation based solely on shell morphology is unreliable, especially in areas where extensive hybridization and introgression is taking place.

In recent years, techniques such as gel electrophoresis, cytology, immunology and artificial hybridization have been used in conjunction with shell morphology in an attempt to resolve the problem of the taxonomic status of *M. galloprovincialis*. The second and major part of this paper reviews the results of such studies. Data from electrophoretic surveys has shown that the two forms of mussel are closely related; genetic identity and genetic distance values are similar to those observed between subspecies of other invertebrates. Cytological and immunological studies on *M. edulis* and *M. galloprovincialis* indicate that the differences between them are small and hardly sufficient to warrant them being classified as separate species. Results from hybridization studies have probably contributed most to a resolution of the problem; there is no evidence of genetic incompatibility between the two forms since the hybrids are fertile and viable backcross F_2 individuals have been produced.

Thus, while the differences between the two forms of mussel are greater than between geographically isolated populations of most species, they are hardly large enough to justify *M. galloprovincialis* being considered a distinct species. The conclusion from this review is that *M. galloprovincialis* cannot be regarded as more than a race or subspecies of *M. edulis* L. and it is suggested that the name *Mytilus edulis* var. *galloprovincialis* be readopted, the ranking suggested by Jeffreys (1863).

Key words: mussels; *Mytilus*; morphological variations; population genetics; cytogenetics; immunology; hybridization; systematics.

INTRODUCTION

The systematic status of *Mytilus galloprovincialis* Lmk. has been the subject of considerable discussion since the 1860s. While some authorities regard it as a distinct species of *Mytilus* others regard it merely as a variety of the larger *Mytilus edulis* L. complex (review, Lubet, 1973).

M. galloprovincialis is believed to have originated in the Mediterranean area (Barsotti & Meluzzi, 1968) but evidence now indicates that it has extended its range northwards onto the Atlantic coasts of W Europe, where it is found intermixed with *M. edulis* in varying proportions (Hepper, 1957; Seed, 1972; Lubet, 1973; Seed, 1974).

The two forms of mussel have been traditionally separated on the basis of shell morphology but in some areas, e.g. the Atlantic coasts of Ireland and NW France where there is evidence of extensive hybridization (Seed, 1972, 1974; Gosling & Wilkins, 1977; Skibinski & Beardmore, 1979; Gosling & Wilkins, 1981), identification on shell characters alone is difficult and even impossible. In recent years the technique of gel electrophoresis (in conjunction with morphological analyses) has been used in an attempt to quantify the genetic differences between the two forms (Ahmad & Beardmore, 1976; Gosling & Wilkins, 1977; Skibinski *et al.*, 1978b; Skibinski *et al.*, 1980; Gosling & Wilkins, 1981).

The first part of this paper discusses the reliability of the morphological criteria used to separate *M. galloprovincialis* from *M. edulis*, while the second part assesses the contribution that gel electrophoresis and other techniques such as cytological, immunological and artificial hybridization studies have made towards a better understanding of the systematics of *Mytilus* in W Europe.

MORPHOLOGICAL CRITERIA USED TO SEPARATE *M. EDULIS* AND *M. GALLOPROVINCIALIS*—AN EVALUATION

Separation of the two forms of mussels is based primarily on external shell contours, internal features of the shell valves and the colour of the mantle edge. Although detailed descriptions of these can be found elsewhere (Lewis & Powell, 1961; Lewis & Seed, 1969; Seed, 1972; Lubet, 1973; Seed, 1974, 1978) a brief account of the reported distinguishing characters will be included at this point.

The shell of *M. galloprovincialis* tends to be higher and flatter than in *M. edulis*, giving distinctly different transverse profiles in the two forms (Fig. 1B). The anterior end of the shell of *M. galloprovincialis* is distinctly beaked or incurved while that of *M. edulis* has a more snub-nosed appearance. In *M. galloprovincialis* the anterior edge of the shell merges smoothly into the dorsal edge, thus giving rise to a rounded convex profile, while that of *M. edulis* is angular where the anterior and dorsal edges meet (Fig. 1A).

Interiorly, the anterior adductor muscle scar is small and circular in *M. galloprovincialis* whereas in *M. edulis* it is narrow and elon-

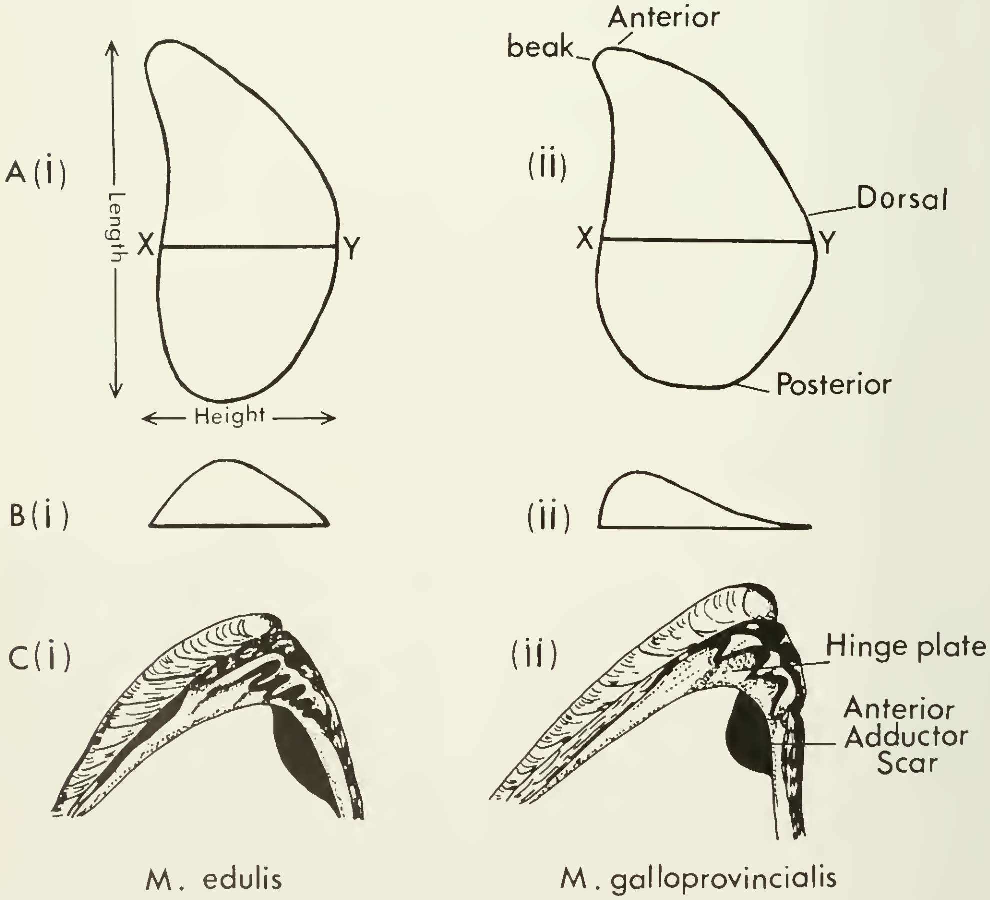

FIG. 1. A (i) and (ii) and C (i) and (ii): some of the salient differences in shell morphology between *M. edulis* and *M. galloprovincialis*. B (i) and (ii): transverse profiles through section XY of a single shell valve of *M. edulis* and *M. galloprovincialis*.

gated. The hinge plate is also smaller in *M. galloprovincialis*, forming a much tighter arc with its rear end much more clearly delimited from the adjacent ventral edge of the valve. The hinge plate in *M. edulis* is a gently curving structure. Both of these characters are illustrated in Fig. 1C. The colour of the mantle edge tends to be deep purple-violet in *M. galloprovincialis* and yellow-brown in *M. edulis*.

Using these characters a number of investigators (Lewis & Seed, 1969; Seed, 1972, 1974) have identified the *M. galloprovincialis* form on the SW coasts of England and on the Atlantic coasts of Ireland and France extending into the English Channel as far as the Cherbourg peninsula. In these areas *M. galloprovincialis* has been found intermixed with *M. edulis* in varying proportions. In some areas, e.g. the Atlantic coasts of Ireland and NW France, separation of the two forms of mussel has proved to be exceedingly difficult due to a considerable degree of overlap in morphological characteristics. This, together with the large numbers of truly intermediate forms observed, suggests that hybridization might be occurring (Seed, 1978).

In other areas, e.g. SW England and the Bay of Biscay (France), the task of separation, although less difficult, was confounded by the fact that shell morphology is so enormously plastic, being influenced by such factors as age and density of mussels, tidal level, and habitat type (Seed, 1968). The effects of these factors appear to be the same for both forms of mussel (Seed, 1978) and this results in a considerable degree of convergence in mixed samples. For example, when the two forms of mussels, together with their hybrids (all spawned artificially) were grown in the wild under identical conditions of temperature, salinity, nutrition, photoperiod and population density, Lubet *et al.* (1984) found that shell shape could not be used to distinguish *M. edulis* from *M. galloprovincialis* or their hybrids.

I will now consider which, if any, of the morphological characters variously attributed to *M. galloprovincialis* are sufficiently constant to distinguish it from *M. edulis* as found on the coasts of Britain, France and Ireland.

External Shell Morphology

Both *M. edulis* and *M. galloprovincialis* exhibit considerable variation in external shell morphology and of the two *M. galloprovincialis* seems to be the more variable form. Seed (1972), in a detailed morphological survey of mussels from sixteen locations on the French coasts, points out that "over 30% of all the mussels examined during this investigation would have been misidentified on external characters alone." Similar problems of identification have been encountered in SW England (Lewis & Seed, 1969). In a survey of Irish mussel populations, Seed (1974) found gross shell morphology to be completely unreliable in separating the two forms; mussels of every conceivable shape were encountered from one locality to another. It would appear, therefore, that overall shell shape in *Mytilus* is so variable, both within and between the two forms of mussels, that it has little if any value in taxonomic studies.

Internal Shell Morphology

The anterior adductor scar and hinge plate size have generally been regarded as more reliable taxonomic characters in the separation of the two forms (Seed, 1978). While the mean adductor scar ratios (adductor scar length/shell length) vary from one locality to another the values tend to be consistently lower in *M. galloprovincialis* than in *M. edulis*. In Ireland, Seed (1974) found the range to be 53–93 for *M. galloprovincialis* and 74–113 for *M. edulis*; in France (Seed, 1972) 41–87 (*M. galloprovincialis*) and 80–124 (*M. edulis*) and at Rock, SW England (Lewis & Seed, 1969) the mean values were 64 ± 10 (*M. galloprovincialis*) and 105 ± 13 (*M. edulis*). However, there are difficulties associated with using this character as the sole means of distinguishing between the two forms. For example, on the west coast of France, Lubet (1959) and Seed (1972) have found considerable overlap in values and distributions were not markedly bimodal; while at sites on the Mediterranean coast—where the *M. edulis* form is very rare—the distribution was distinctly skewed. The percentage misidentification using this character alone was as high as 45% for areas on the west coast of France and over 60% for several localities in the Mediterranean Sea.

Like the adductor scar ratios, the hinge plate ratios (hinge plate length/shell length)—although difficult to measure accurately (Lewis & Seed, 1969)—tend to be lower in *M. galloprovincialis* than in *M. edulis*: 74–77 (*M. galloprovincialis*) and 70–115 (*M. edulis*) in Ireland; 51–70 (*M. galloprovincialis*) and 56–99 (*M. edulis*) in France; 56 ± 8 (*M. gallopro-*

vincialis) and 72 ± 9 (*M. edulis*) at Rock. Once again there are difficulties with the use of this character as a sole means of distinguishing between the two forms because the degree of overlap is such that for the majority of French sites (Seed, 1972) and for Rock (Lewis & Seed, 1969) the frequency distributions of the hinge plate ratios were generally unimodal for mixed samples of the two forms of mussel. In the Mediterranean, 33% of mussels from Sète would have been identified as *M. edulis* using this character alone.

Because hinge plate size has limited usefulness as a means of separating mixed populations, Seed (1972) suggests that hinge plate *shape* should also be considered. But the shape of the hinge plate is a difficult character to assess, especially in upper shore mussels where the shape of the anterior end is obscured by erosion, shell thickening and divergence of umbones (Lewis & Seed, 1969).

In contrast to the situation observed in W France the hinge plate ratios in Irish samples generally proved to be a more reliable taxonomic character than the adductor scar ratios, with frequency distributions in some cases quite distinctly bimodal (Seed, 1974). The percentage of mussels misidentified using hinge plate ratios was considerably lower than for adductor scar ratios, i.e. 14% and 50% respectively for an exposed site (Portstewart) on the N coast of Ireland.

Mantle Edge Colour

This character has been cited by Seed (1972) as being generally a reliable taxonomic character for French populations of mussels. This is surprising in view of his comment that "mantle edge colour varied from almost white in some individuals through all shades of brown, reddish-brown and purple to deep-violet in others." In addition, in the Concarneau-Les-Sables region on the W coast of France between 20–45% of the populations would have been misidentified using mantle edge colour alone. More remarkably still, the value of this character completely breaks down in samples from Banyuls, Sète and Riou on the Mediterranean coast of France where over 60% of mussels would have been identified as *M. edulis* using this character! In SW England, Lewis & Powell (1961) observed dark mantle types with typical *M. edulis* characters at all sites, while in Ireland Seed (1974) observed "individuals with characteristic *galloprovincialis*-like shells with mantles that were distinctly pale in colour." Certainly in Ireland the value of mantle colour as a diagnostic taxonomic character is questionable since at 18 of the 37 Atlantic sites sampled more than 20% of individuals were misidentified using this character and at some sites the misidentification percentage was as high as 46%.

In conclusion, there is no single character which can be reliably used to separate the two forms of mussels in W Europe. In some instances, e.g. at Rock, it appears that using a combination of characters, such as adductor and hinge plate ratios together with mantle edge colour, gives a high degree of accuracy. Moreover, at Rock, morphological and anatomical differences were also accompanied by marked differences in breeding patterns, peacrab infestation and growth potential (Seed, 1971). Sites such as Rock, where the two mussel types are quite distinctive are of especial interest and obviously require further study. Lewis & Seed (1969), using a combination of morphological and anatomical characters at a number of other sites in SW England did not, however, produce identical results (see Table 1). The same inconsistencies were observed by Seed (1972) for mixed populations of the two forms of mussels in W France. Furthermore, in areas such as the Atlantic coasts of Ireland and NW France, where hybridization is taking place, the large number of intermediate forms makes accurate identification of *M. galloprovincialis* and *M. edulis* an almost impossible task especially at exposed locations (Seed, 1974).

THE CONTRIBUTION OF ELECTROPHORESIS, CYTOLOGY, IMMUNOLOGY AND ARTIFICIAL HYBRIDIZATION TO THE SYSTEMATICS OF *MYTILUS*

Electrophoretic Studies

Morphological studies do not fully take into account that the environment can substantially influence the morphological characteristics of a given species. Therefore, systematic information that is relatively free of environmentally-induced changes is highly desirable. Protein electrophoresis together with techniques such as DNA-RNA hybridization, immunology and amino acid sequencing

TABLE 1. A comparison of identifications based on mantle colour, overall shell shape and hinge and adductor scar ratios in samples of *Mytilus* from SW England (after Lewis & Seed, 1969).

		Method of identification						
		Mantle colour		Shell shape			Hinge and add. scar	
Locality	Sample size	*gall.*	*edulis*	*gall.*	?	*edulis*	*gall.*	*edulis*
Penzance Harbour	20	9	11	2	5	13	6	14
St. Ives	62	62	—	25	10	27	53	9
Bude low shore	44	44	—	11	20	13	36	8
Bude high shore	70	?	?*	18	11	41	52	18

*Mantle colour appeared too dark for *M. edulis* and not purple enough for *M. galloprovincialis*.

have proved invaluable in quantifying genetic differences between species. For comparisons of closely related species, electrophoresis has proved to be a most efficacious technique (see Ferguson, 1980 for review and references). "In most cases where the status of a 'species' is in dispute," as is the case for *M. galloprovincialis*, "an electrophoretic investigation of enzyme loci is likely not only to produce an answer less open to argument than one based on conventional morphological criteria, but it is able to produce results more quickly" (Thorpe *et al.*, 1978).

Genetic variability at several enzyme loci has been surveyed in allopatric populations of pure *M. edulis* from approximately 75 sites on the coasts of Ireland and France (Gosling & Wilkins, 1977, 1981), Britain (Ahmad *et al.*, 1977), and Denmark (Theisen, 1978). In general, there appears to be very little geographic variation in allele frequency with the exception of the Baltic area where Theisen (1978) has observed conspicuous differences in allele frequency at three enzyme loci between the North Sea and Kattegat *M. edulis* and those from the Baltic. Salinity is believed to be the selective agent maintaining these differences since the maximum change in allele frequency, at the three loci, roughly coincides with the transition from the rather stable low salinities of the Baltic (7–10‰) to the higher more variable salinities of the Kattegat (22–30‰).

In contrast, pure populations of *M. galloprovincialis* have been analysed electrophoretically from only a small number of sites (about four) in the Mediterranean and Black Sea. Allele frequency data from 12 enzyme loci (Skibinski *et al.*, 1980) at two sites (Venice and Gibraltar) indicate that there are large differences in frequency at several loci (Aminopeptidase (*Ap*), leucine aminopeptidase-1 (*Lap-1*), phosphoglucomutase (*Pgm*), and phosphoglucose isomerase(*Pgi*). However, Nei's genetic identity value (Nei, 1972) for the two populations is 0.976, which is in the range expected for conspecific populations. Although genetic identity values have not been published for *M. edulis* populations the indications are that they would be similar (if not higher) than the above value for *M. galloprovincialis*. Nei's (1972) genetic identity ($\bar{I}$) and genetic distance ($\bar{D}$) values for comparisons between *M. edulis* from S Wales and *M. galloprovincialis* from Venice were found to be 0.842 and 0.167 respectively (Skibinski *et al.*, 1980). These values are similar to the mean values observed for comparisons between subspecies of other invertebrate taxa (Snyder & Gooch, 1973; Ayala *et al.*, 1974; Avise, 1976). Large $\bar{I}$ values (>0.90) have been observed, however, for comparisons of distinct species of *Elliptio* (Davis *et al.*, 1981), *Drosophila* (Sene & Carson, 1977), *Partula* (Johnson *et al.*, 1977) and *Cerion* (Gould & Woodruff, 1978), thus illustrating the dangers associated with inferring taxonomic relationships from $\bar{I}$ or $\bar{D}$ values alone. Using a band-counting electrophoretic method (see Ferguson, 1980 for description of technique) ten individuals of *M. edulis* (W Ireland) and *M. galloprovincialis* (S France) have been compared on a single gel; the coefficient of similarity between them was 0.809 (Gosling & King, unpublished results), a value very close to that observed by Skibinski *et al.* (1980)

TABLE 2. Allele frequencies at *Est-D, Lap-1* and *Pgi* for *M. edulis* at Mumbles, S Wales and *M. galloprovincialis* at Venice. E and G are the two compound alleles at each locus. Alleles are numbered in order of increasing anodal mobility. N = number of individuals analysed. Data from Skibinski *et al.* (1980).

	N	1	2	3	4	5	6	7
Est-D		G			E			
M. edulis	1286	0.005	0.024	0.001	0.941	0.004	0.020	0.003
M. gall.	120	0.038	0.950	0.0	0.013	0.0	0.0	0.0
Lap-1		E				G		
M. edulis	1286	0.019	0.198	0.724	0.006	0.049	0.004	0.0
M. gall.	31	0.0	0.0	0.032	0.032	0.468	0.043	0.032
Pgi		G				E		
M. edulis	2005	0.013	0.033	0.247	0.049	0.599	0.048	0.011
M. gall.	226	0.009	0.022	0.801	0.142	0.024	0.0	0.022

using allele frequency data from 16 enzyme loci. Sarich (1977) has found positive agreement between the two methods for interspecific comparisons of *Dipodomys* (kangaroo rat).

The two forms of mussel differ most at the following enzyme loci: Esterase-D (*Est-D*), *Lap-1, Pgi, Ap, Lap-2* and *Pgm* with the first three being partially diagnostic (a locus is considered to be diagnostic if an individual can be assigned to the correct species (or form) with a probability >0.99 (Avise, 1974)). The search for diagnostic loci, where the allele frequency distributions do not overlap, has to date been unsuccessful (Skibinski *et al.*, 1978b). Table 2 presents typical allele frequency data at the *Est-D, Lap-1* and *Pgi* loci for *M. edulis* (S Wales) and *M. galloprovincialis* (Venice). One must be cautious however about accepting that 'typical' frequencies for *M. galloprovincialis* exist since so few populations have been analysed and since moderate to large differences in allele frequency have been observed at several loci (see above). The allele frequency distributions at the three loci are significantly different between the two mussel types; the differences are greater at the *Est-D* and *Lap-1* loci than at the *Pgi* locus. Skibinski *et al.* (1978b) have suggested combining alleles at each of these loci to form compound (synthetic) alleles called E and G. The Est^E (alleles 4, 5, 6 and 7), Lap^E (alleles 1, 2, 3 and 4) and Pgi^E (alleles 5 and 6) compound alleles combine those alleles at high frequency in *M. edulis* while the Est^G (alleles 1, 2 and 3), Lap^G (5, 6 and 7) and Pgi^G (1, 2, 3 and 4) compound alleles combine those at high frequency in *M. galloprovincialis* (see Table 2). This procedure smooths out allele frequency differences between populations of *M. edulis* or *M. galloprovincialis.* For example, the frequencies of alleles 3 and 4 at the *Pgi* locus were 0.544 and 0.344 respectively in *M. galloprovincialis* from Gibraltar and 0.801 and 0.142 in *M. galloprovincialis* from Venice. However, the frequency of the compound allele G is 0.951 for Gibralter and 0.974 for Venice.

In summary, electrophoretic investigations on allopatric populations of the closely related *M. edulis* and *M. galloprovincialis* have revealed little geographic heterogeneity in allele frequency in *M. edulis* but a moderate amount in *M. galloprovincialis.* When the two forms are compared they differ significantly in allele frequency at the *Est-D, Lap-1, Pgi, Ap, Lap-2* and *Pgm* loci with the first three being partially diagnostic.

This information has been used to study the genetic structure of mixed populations of the two forms of mussels at British and Irish coastal sites. In Britain, Hepper (1957) reported the presence of a form of mussel, "the Padstow mussel"—which corresponded closely to the description of *M. galloprovincialis*—on the coasts of SW England. Later investigations (Seed, 1971) indicated that the two forms of mussels in that area were morphologically quite distinct, had different spawning cycles, growth rates and infection rates with *Pinnotheres pisum* (pea-crab); on the basis of these differences Seed sug-

TABLE 3. Allele frequencies at *Pgi* in *M. edulis* (Group 1) and the "Padstow mussel" (Group 2) from Rock, SW England (data from Ahmad & Beardmore, 1976). Data from Skibinski *et al.* (1980) have been included for comparative purposes.

		G				E		
	N	1	2	3	4	5	6	7
M. edulis (S Wales)	2005	0.013	0.003	0.247	0.049	0.599	0.048	0.011
Group 1	100	0.005	0.070	0.365	0.125	0.410	0.025	0.0
Group 2	100	0.025	0.035	0.460	0.390	0.080	0.010	0.0
M. gall. (Venice)	226	0.009	0.022	0.801	0.142	0.024	0.0	0.022

gested that the two types of mussel should be regarded as distinct species of *Mytilus*. At this stage no firm genetic evidence had been gathered on the two forms of mussels but the morphological and physiological differences suggested that there were good grounds for biochemical investigations.

Ahmad & Beardmore (1976), using the morphological characteristics already discussed, separated mussels from Rock, S.W. England, into *M. galloprovincialis* and *M. edulis* forms and assayed each group for *Pgi*, *Pgm*, *Lap-2* and *Ap* (the electrophoretic techniques for *Est-D* and *Lap-1* had not yet been developed). The results indicated significant differences in allele frequency between the two groups at the *Pgi*, *Ap* and *Lap-2* loci but not at the *Pgm* locus. On the basis of these differences, together with the close similarity in allele frequency between the *M. galloprovincialis* form at Rock and *M. galloprovincialis* from the Mediterranean, the authors concluded that the "Padstow mussel" was indeed *M. galloprovincialis*. In addition, they felt that the two groups at Rock were genetically discrete and reproductively isolated entities since no genetic evidence for hybridization was observed. Allele frequencies at the *Pgi* locus (the two groups showed the biggest difference in frequency at this locus) for the two types of mussel are presented in Table 3. Allele frequency data for *M. edulis* (S Wales) and *M. galloprovincialis* (Venice) have been included for comparative purposes (data from Skibinski *et al.*, 1980). While the *M. edulis* and *M. galloprovincialis* group differed significantly at the *Pgi* locus it would appear from Table 3 that there are large differences in allele frequency between *M. edulis* from Rock and *M. edulis* from S Wales. Ahmad & Beardmore (1976) suggested that in British populations of *M. edulis* there may be a N–S cline in the frequency of Pgi^3 and Pgi^5. However, subsequent analysis has indicated little geographic heterogeneity in allele frequency at the *Pgi* locus among several British populations of *M. edulis* (Ahmad *et al.*, 1977). It may be that the *M. edulis* group was not a homogeneous group but contained some *M. galloprovincialis* forms. Ahmad & Beardmore (1976) did have some difficulties in the separation of the two forms of mussel as the following illustrates. The remainder of the sample from Rock (187 individuals) was assayed for *Pgi* and allele frequencies resembled—but were not identical—to those observed for the *M. galloprovincialis* group (using the compound alleles E and G one can compare the number of E/G heterozygotes observed with Hardy-Weinberg expectations (see later); there is a significant deficiency of heterozygotes ($P < 0.001$) in this sample which indicates that it is in fact a mixture of populations with differing allele frequency). After electrophoretic analysis Ahmad & Beardmore (1976) examined the 187 individuals morphologically and found that the majority (155) resembled the 'Padstow mussel' (Group 2) but 32 individuals could not be classified at all. However, genetically they were similar to *M. edulis* (Group 1). This illustrates once more the difficulties of distinguishing between these two forms of mussels using morphological criteria.

The Rock site was revisited by Skibinski *et al.* (1978a), and mussels were collected from an area close to the one sampled by Ahmad & Beardmore (1976). In addition, mussels were collected from an exposed rocky shore at Croyde, N Devon. The samples were divided into *M. galloprovincialis*, *M. edulis* and intermediates but this time a scoring system for the individual morphological characters was used with typical *M. edulis* getting low scores, typical *M. galloprovincialis* high scores and the intermediates scoring between the two extremes. However, there were difficulties with the scoring method. The authors observed "some phenotypic overlap for individual morphological characters between pure *M. edulis* and pure *M. galloprovincialis* populations (from Ilfracombe, N. Devon and Venice) so intermediate scores *are expected* [my italics] in the absence of hybridization." The following proportions were observed at the two sites:

	% *M. edulis*	Intermediates	*M. galloprovincialis*
Rock	1	14	85
Croyde	65	33	2

Each group was assayed for the three partially diagnostic loci and significant differences in allele frequency at the three loci were observed between *M. galloprovincialis* and *M. edulis* at both sites. Allele frequency for the intermediates fell between the frequencies observed for the extremes (intermediates this time appear to be truly intermediate and not *M. edulis* individuals as observed by Ahmad & Beardmore (1976)). In general, the frequencies of *M. edulis* and *M. galloprovincialis* corresponded with those observed for allopatric populations of the two forms. This was particularly true for Rock *M. edulis* which on this occasion were very similar in allele frequency at the *Pgi* locus to *M. edulis* from S Wales. This probably reflects better separation of the two forms using a scoring system.

As described above, the frequencies of alleles at each locus were pooled to form compound alleles E and G for the total sample of mussels from each site. Analysis of the data revealed:

a) A large and significant deficit of E/G heterozygotes compared with Hardy-Weinberg expectations in both samples, which was to be expected when sampling a mixture of populations, each with differing allele frequency.

b) A significant excess of triple homozygotes in the Rock sample and a significant excess of double homozygotes in the sample from Croyde (*Pgi* was not studied at Croyde). This occurs because the frequency of $Est\text{-}D^E$ / $Est\text{-}D^E$ $Lap\text{-}1^E$ / $Lap\text{-}1^E$ Pgi^E / Pgi^E homozygotes covary positively between contributing populations; the same holds true for G/G homozygotes.

c) An overall excess of triple heterozygotes at Rock and an excess of double heterozygotes at Croyde; the excess was higher among morphologically intermediate mussels. This has been interpreted as evidence for hybridization since "If at a locus the alleles of highest frequency are different in two species, a population containing a significant number of F_1 hybrids will show an excess of heterozygotes compared with the expectation for either species or for a mixed population of the two pure species. The excess will be relatively greater for multiple heterozygotes at two or more diagnostic or discriminatory loci" (Skibinski *et al.*, 1978a). The amount of hybridization was calculated as 2% for Rock and 5% for Croyde. In addition, a significant number (21%) of 'F_2' and 'backcross' genotypes were observed in the intermediate group at Croyde but not at Rock.

Subsequently, Skibinski *et al.* (1978b) investigated mussel populations at Robin Hood's Bay (RHB) on the NE coast of England and at King's Dock in S Wales. The data from the Croyde site was also included in that paper. Mussels were again separated on the basis of the morphological criteria outlined by Lewis & Seed (1969) and the same scoring system as before was used. Once again difficulties were encountered in identifying *M. galloprovincialis* and *M. edulis* individuals. At Croyde and RHB typical *M. galloprovincialis* and *M. edulis* were identified (this was the first report of *M. galloprovincialis* in the North Sea) with the best discriminating characters being the colour of the mantle edge and the size and shape of the adductor scar. However, intermediates and individuals showing all combinations of *M. edulis* and *M. galloprovincialis* characteristics were present. These two characters were of little use at King's Dock and while most of the mussels at that site were identified on other characteristics as *M. galloprovincialis*, they had a large adductor scar and the yellow mantle edge of the *M. edulis*. It is not surprising that Skibinski *et al.* (1978b) felt that "quantification of the morphological variation within and between populations is certainly desirable"! The morphological extremes—which represented a much smaller fraction (5–26%) of the total sample in each case—were typed for *Est-D* and *Lap-1* and the data were treated in the same way as described in Skibinski *et al.* (1978a)—see above. The results for the three sites are summarized in Table 4. Skibinski *et al.* (1978b) conclude from these results that the extent of mixing is low at Croyde (but see above), intermediate at RHB and high at King's Dock. It would appear from the genetic

TABLE 4. Summary of results for *Lap-1* and *Est-D* in mixed samples of mussels from Croyde, Robin Hood's Bay (RHB) and King's Dock [data from text of Skibinski *et al.*, 1978b]. ** = P < 0.01; *** = P < 0.001; n.s. = not significant.

	Croyde	RHB	King's Dock
Allele frequency differences between morphological extremes	Large	Smaller than Croyde	None
Heterozygote deficits			
Lap-1	***	***	n.s.
Est-D	***	**	n.s.
Genotype Association			
Excess of double homozygotes	***	***	n.s.
Excess of double heterozygotes	***	n.s.	n.s.

evidence so far that at all of the sites where the two forms of mussel were found together, hybridization and introgression to varying extents were taking place between them.

Let us now consider the situation in Ireland. In 1974, Seed made extensive collections of *Mytilus* from various localities around the Irish coasts, a total of 43 sites in all. He found the *M. galloprovincialis* form intermixed with *M. edulis* in varying proportions on the Atlantic coasts of Ireland but found no evidence for the occurrence of *M. galloprovincialis* along the east coast (Irish Sea Coast). At all Atlantic sites there was a considerable degree of overlap in morphological characters and the greatest number of intermediate forms were encountered on exposed shores.

A preliminary report (Gosling & Wilkins, 1977) on allele frequency at the *Pgi* locus in populations of *Mytilus* (approximately twenty sites) on Irish coasts indicated that mussels on the Irish Sea coast differed significantly in allele frequency from Atlantic exposed and sheltered shore mussels. Subsequent investigations using the *Lap-2* and *Pgm* loci supported this observation (Gosling & Wilkins, 1981). Mussels on the Irish Sea coast exhibited allele frequencies at the three loci which were very similar to populations of 'pure' *M. edulis* from the coast of France (Gosling & Wilkins, 1977, 1981) and Britain (Ahmad *et al.*, 1977). Allele frequencies were homogeneous over localities; there were no significant deviations from Hardy-Weinberg expectations at the three loci and the evidence suggested that mussels in this area constituted a single panmictic population of *M. edulis* alone. On the Atlantic coasts of Ireland there were marked differences in allele frequency at all loci between exposed and sheltered shore mussels and large significant deficiencies of heterozygotes at the *Pgi* locus were observed at all but one of the exposed sites while the majority of the sheltered shore samples did not exhibit such deficiencies. Heterozygote deficiencies were not correlated with absolute or relative size of individual mussel nor with position of the mussels on the shore. The indications were—from analyses of *M. galloprovincialis* from the Mediterranean and Northern France (Gosling & Wilkins 1977, 1981)—that the exposed shore samples, and to a much lesser extent the sheltered shore samples constituted a mixture of interbreeding *M. galloprovincialis* and *M. edulis* (Table 5).

No attempt was made to separate the two forms of mussel on exposed shores. However, at Lough Ine, a sheltered site on the S coast of Ireland, where previous workers (Baird, cited by Hepper, 1957; Kitching *et al.*, 1959; Seed, 1974) have reported a high incidence (58–77%) of the *M. galloprovincialis* form, mussels exhibited allele frequencies at the three loci which were very similar to exposed shore frequencies. Subsequently *M. edulis*-like individuals (approximately 33%) were separated from the sample but no statistical differences in allele frequency were observed at any of the three loci between these individuals and the remainder of the sample. Interestingly, the *M. edulis* group, in contrast to the total sample, did not exhibit significant deficiencies of heterozygotes at the *Pgi* locus relative to Hardy-Weinberg expectations.

It would appear therefore that in Ireland, at least, the *M. galloprovincialis* form occurs mainly at exposed sites, whereas *M. edulis* is more common in sheltered shore environments. Skibinski *et al.* (1983), using *Est-D* allele frequency data, have confirmed our

TABLE 5. Mean frequency of the major *Pgi*, *Lap-2* and *Pgm* alleles in pure *M. edulis* from San Vaast, N France, in Irish coastal mussels and in pure *M. galloprovincialis* from two sites in the Mediterranean (Cannes and Venice). Data from Gosling & Wilkins (1981). Allele numbers correspond to those in Ahmad & Beardmore (1976).

		Pgi					*Lap-2*				*Pgm*		
	N	1	3	4	5	N	2	3	4	N	2	3	4
M. edulis France	92	0.04	0.26	0.0	0.64	102	0.14	0.66	0.20	104	0.12	0.66	0.22
Mytilus Irish Sea	527	0.05	0.30	0.0	0.61	457	0.11	0.61	0.26	527	0.13	0.64	0.21
Atlantic sheltered	2219	0.05	0.37	0.02	0.52	2078	0.10	0.57	0.30	2137	0.13	0.60	0.25
Atlantic exposed	1138	0.04	0.44	0.15	0.29	1138	0.09	0.48	0.41	1138	0.14	0.57	0.28
M. gall. Mediterranean	154	0.01	0.83	0.13	0.02	149	0.05	0.52	0.43	68	0.10	0.53	0.34

observation. Unfortunately, a similar analysis has not been possible for British sites due to insufficient information on the degree of exposure of the sites investigated there. However, as Skibinski *et al.* (1983) point out "exposure score alone cannot be used to predict allele frequency" since at several sheltered sites on the Atlantic coasts of Ireland (Gosling & Wilkins, 1981) allele frequency and genotype proportions resembled those observed for exposed shore mussels. Also, mussels at the single exposed site sampled on the Irish Sea coast (Gosling & Wilkins, 1981) did not differ appreciably in allele frequency from the sheltered shore samples in the same area and did not exhibit deficiencies of heterozygotes at the *Pgi* locus. Skibinski *et al.* (1983) have also observed low frequencies of $Est\text{-}D^2$ (a diagnostic allele for *M. galloprovincialis*) at moderately exposed sites on the Irish Sea coast of Ireland.

The results for Irish sites are interesting in the light of an earlier report by Murdock *et al.* (1975). These authors observed a significant correlation between frequencies of the common alleles at a *Lap* locus and exposure in samples of *Mytilus* from Irish coasts (It is not clear whether the *Lap* locus studied by Murdock *et al.* (1975) is *Lap-1*; examination of their allele frequency data suggest that it is not). This paper has been much cited as evidence for selection in mussel populations. The correlation between allele frequency and exposure may not in fact be a correlation with exposure *per se* but may reflect the higher incidence of *M. galloprovincialis* alleles at the more exposed sites investigated by Murdock *et al.* (1975).

The present distribution of *M. galloprovincialis* in W Europe may be inferred by using allele frequency data from the three best discriminatory loci. Fig. 2 illustrates that *M. galloprovincialis* is present on the SW peninsula of Britain and at a single location on the S coast of Wales (Skibinski & Beardmore, 1979); on the S, W and N coasts of Ireland (Gosling & Wilkins, 1977, 1981; Skibinski & Beardmore, 1979) on the NE coast of England, on the NW coast of Scotland (Skibinski & Beardmore, 1979) and as far N (60° N) as the Shetland and Orkney islands (Gosling, unpublished results). The distribution of *M. galloprovincialis* has been both confirmed and extended using electrophoretic data, with one exception: Skibinski & Beardmore (1979) found no evidence of *M. galloprovincialis* on the S coast of Wales outside of the King's Dock site. However, Hepper (and others cited by him) in 1957, using general shell shape and mantle edge colour as morphological criteria, found *M. galloprovincialis* at several locations on the S coast and at places, e.g. Cardiff; the proportion of *M. galloprovincialis* recorded was 60%. Either the morphological characters used by Hepper are worthless (and this may be true) or else both have sampled different sites on the S Wales coast. It is difficult to check this since location names have not been given for the sites sampled by Skibinski & Beardmore (1979).

The electrophoretic evidence suggests that where *M. galloprovincialis* and *M. edulis* are

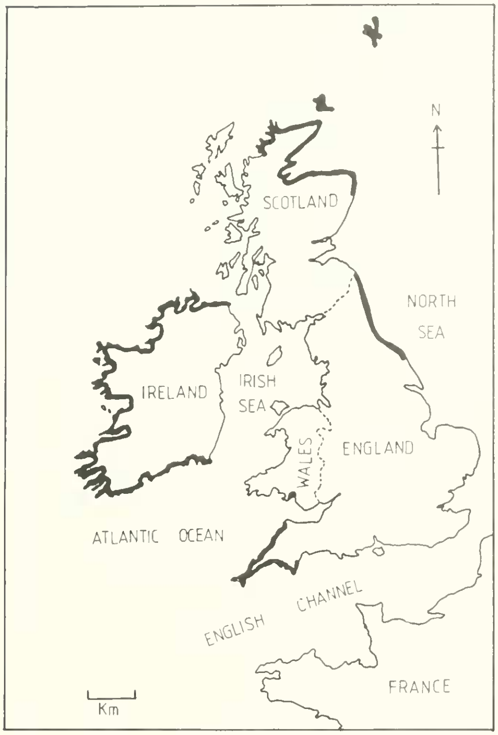

FIG. 2. Map showing in heavy outline the geographical areas in which the *M. galloprovincialis* form has been detected using allele frequency data from the partially diagnostic loci *Pgi*, *Est-D* and *Lap-1*.

found together they are at least interbreeding and in some areas intergradation between the two forms is extensive. The extent of intergradation in mixed samples of mussels from British (16 sites) and Irish (9 sites) has been assessed by measuring deviations from Hardy-Weinberg expectations and genotypic correlations between *Est-D* and *Lap-1*. Three statistics have been used: F (Wright, 1951), which measures deviations from Hardy-Weinberg equilibrium, R1 which measures the strength of association of homozygote genotypes and R2 which measures the excess of double heterozygotes expected. General trends in the values of these statistics occur as a result of interbreeding: on hybridization F decreases, R2 increases and R1 remains unaltered and if intergradation proceeds, all three measures decrease at different rates to zero when the process is complete (Skibinski & Beardmore, 1979). In SW England hybridization but little intergradation is occurring between the two forms of mussel; at other localities, e.g. on the S coast of Ireland, intergradation, though not complete, is considerable. In general, the extent of intergradation is greater in Ireland, Scotland and NE England than in SW England.

In summary, electrophoretic analysis of allopatric populations of *M. edulis* and *M. galloprovincialis* have shown that the two forms are closely related; genetic identity and genetic distance values for comparisons between the two mussel types are similar to the mean values observed by various workers for comparisons between subspecies of other invertebrate taxa. In areas where the two forms are found together, e.g. the coasts of Britain and Ireland, electrophoretic investigations have enabled us to quantify the extent of hybridization and intergradation taking place between *M. edulis* and *M. galloprovincialis* in these areas. In addition, we now have more precise information on the micro- and macrogeographic distribution of the two forms in W Europe. However, while these results are interesting in themselves, they do not resolve the problem: is the *M. galloprovincialis* form a distinct species of *Mytilus* or is it merely a race or ecotype of its progenitor *M. edulis*?

Nearly all the most illuminating investigations of speciation have been those where a number of techniques have been employed. With this in mind, information from cytological, immunological and artificial hybridization studies is presented and discussed in the following section in an attempt to explore further the systematic relationship between the *M. edulis* and *M. galloprovincialis* forms.

Cytological Studies

The study of karyotypes has been used as a valuable complement to biochemical methods of identifying species, hybrids and more rarely, populations (Chevassus *et al.*, 1978). Karyotypes have been described for *M. edulis* (Ahmad & Sparks, 1970; Thiriot-Quiévreux & Ayraud, 1982; Moynihan & Mahon, 1983; Thiriot, this volume) and *M. galloprovincialis* (Thiriot-Quiévreux & Ayraud, 1982; Thiriot, this volume). While these authors agree that the two forms exhibit a diploid chromosome number of 28, their reports are at variance as to the karyotype of these two mussels. Ahmad & Sparks (1970), examining *M. edulis* from the W coast of North America, observed three pairs of acrocentric and a variable number of metacentric and submetacentric chromosome pairs. They also observed structural chromosomal polymorphism within populations of *M. edulis* (and *M. californianus* Conrad) which they attributed to the presence of pericentric inversions or centromere shifts in the autosomes. Moynihan & Mahon (1983) observed 5–6 metacentric pairs, 6–8 submetacentric pairs and 0–3 subtelocentric pairs in *M. edulis* from Galway, on the W coast of Ireland (there was no evidence of the *M. galloprovincialis* form at this site [Gosling & Wilkins, 1981]). Moynihan & Mahon (1983) attributed the variation in the number of chromosome types to differential contraction of chromosomes rather than to any structural changes which could affect centromere position, e.g., pericentric inversions or non-reciprocal translocations. Thiriot-Quiévreux & Ayraud (1982) and Thiriot (this volume) have observed 2 pairs of metacentric, 6 pairs of submetacentric and 6 telocentric pairs in French populations of *M. edulis* from Charron ('pure' *M. edulis*) and Brest (mixture of *M. edulis* and *M. galloprovincialis*). Three populations of *M. galloprovincialis* were also analysed from Banyuls and Villefranche ('pure') and Brest (mixed). *M. edulis* from 'pure' and mixed populations differed consistently from *M. galloprovincialis* in that chromosome pair number 2 was metacentric in *M. edulis* but telocentric in *M. galloprovincialis.* This difference was ascribed to a pericentric inversion. Thiriot-Quiévreux & Ayraud (1982) concluded that the difference in the position of the centromere between *M. edulis* and *M. galloprovincialis* was further evidence that these two forms should be regarded as separate species.

Since certain types of chromosomal rearrangements, e.g. inversions and translocations, of various kinds may play a determining role in the speciation process (but see Zouros, 1982) in many groups of animals has been repeatedly suggested by White (1968, 1978). It is believed that such inversions could provide the raw material for the forces of speciation which would eventually convert these polymorphisms within the ancestral population into a system of fixed inversions in two lineages. While this may be an attractive hypothesis to explain the chromosomal differences observed between the two mussel types it must be treated with caution for the following reasons:

1. There appear to be considerable technical difficulties with karyotypic analysis in *Mytilus,* as evidenced by the lack of congruence between the results obtained by various investigators.
2. To date, very few allopatric and sympatric populations of *M. edulis* and *M. galloprovincialis* have been analysed cytologically.
3. Polymorphisms for pericentric inversions have been observed within and between populations of *M. edulis* itself (Ahmad & Sparks, 1970).

Karyotypic changes are *not* universal for speciation; for example, homosequential species complexes have been observed in *Drosophila (D. mulleri, D. aldrichi* and *D. wheeleri*). On the other hand, certain ecological preferences can be tied up with chromosomal polymorphism within a single species, e.g. the marine snail *Nucella lapillus,* where a polymorphism for chromosome number has been observed (Staiger, 1955). Populations on exposed shores exhibit a diploid chromosome number of 26 while those on sheltered shores have 36 chromosomes. Heterogeneous colonies with intermediate chromosome frequencies (26–36) have been observed in intermediate localities.

In conclusion, there is no valid reason, on the basis of a single pericentric inversion, to consider *M. galloprovincialis* as a distinct species of *Mytilus.* Further work on allopatric and sympatric populations of the two forms is in progress (C. Thiriot and D. Dixon, personal communications, 1982). In addition, the karyotypic analysis of wild, e.g. exposed shore populations, and artificially-produced 'hybrids' between *M. galloprovincialis* and *M. edulis* is also underway (C. Thiriot and P. Lubet, personal communications, 1982).

Immunological Studies

Serological methods are relatively free of environmentally-induced changes, being based on the quantitative precipitation of proteins. Moreover, when the precipitations are performed in gel-immunodiffusion or immunoelectrophoresis the sensitivity of serological methods is enhanced and even the rate of cross-reactivity between individuals very close on the zoological scale can be appreciated. Such an approach has been used by Bisignano *et al.* (1980) to compare the muscle protein antigens extracted from samples of *M. galloprovincialis* (Sicily) and *M. edulis* (location not stated). These authors observed that all of the antigens present in *M. galloprovincialis* were present also in *M. edulis* and that *M.*

edulis possessed one extra antigen not observed in *M. galloprovincialis*. Also, one antigen present in *M. edulis* was present only at low concentrations in *M. galloprovincialis*. On the basis of these results the authors concluded that *M. galloprovincialis* was indeed a distinct species derived from *M. edulis*.

Since there is little published information on immunological comparisons between conspecific populations, congeneric species or species of different genera of molluscs it is difficult to evaluate the results of Bisignano *et al.* (1980). Two reports, however, are worth discussing. Investigating the genetic similarities between the closely related cockle species *Cardium edule* and *C. glaucum* using crossed immunoelectrophoresis, Brock (1980) observed seven species-specific antigen-antibody reactions between the two species. It is unlikely that these reflect intraspecific variation, as the same compatibility/noncompatibility patterns were observed for different populations of the two species, both allopatric and sympatric. The high compatibility between antigens of *C. edule* and *C. glaucum* led Brock to conclude that these are closely related; the discovery of seven species-specific antigens indicated that they should, however, be regarded as distinct species. This agrees with ecological (Brock, 1979) and genetic (Gosling, 1980) evidence to date. Additional investigations on a species of the same genus, *C. echinatum* (believed by some authorities [Bowden & Heppell, 1966; Fischer-Piette, 1977] to belong to the genus *Acanthocardia*), showed 17 and 19 species-specific antigens for the *C. echinatum C. glaucum* and *C. echinatum C. edule* comparisons respectively.

Davis & Fuller (1981) have carried out an impressive immunological survey on 52 species belonging to 27 genera of North American Unionacea, the object being to establish a classification based on the immunology, morphology, fossil record and zoogeograpy of this large group of freshwater bivalves. Cross comparisons between species pairs of the same genus (*Elliptio, Anodonta, Fusconaia, Margaritifera, Quadrula* and *Lampsilis*) indicated that they differed by one to six antigens with most species pairs differing by more than two antigens. When species of different genera were compared the average antigen difference between them was approximately six. These values are lower than those observed by Brock for species of *Cardium*. This is not unexpected since the relationship between immunoelectrophoretic genetic distance and taxonomic hierarchy—presumably based on comparative morphology—is not a simple one. There is in fact no direct correspondence (Davis & Fuller, 1981) except in cases where protein and organismic evolution both have proceeded in a regular fashion with regard to elapsed time (Ferguson, 1980). This further emphasizes the desirability of applying a multi-pronged approach to taxonomic problems.

In light of the above reports, what inferences can be drawn from the results of Bisignano *et al.* (1980) for the two forms of *Mytilus*? On the basis of one antigen difference they could be regarded as separate species (Davis & Fuller, 1981). On the other hand, the differences observed by Bisignano *et al.* (1980) could be simply intraspecific variation since, apparently, only one population of *M. edulis* was compared with one of *M. galloprovincialis*. Further work is necessary in order to determine whether immunological differences persist when allopatric and sympatric populations of the two forms are analysed. Brock (personal communication, 1982) used crossed immunoelectrophoresis but was unable to distinguish two types of mussels in mixed samples from Irish coasts. Immunological investigations on sympatric populations of the two forms of mussels from areas such as SW England, where morphological, genetic and physiological differences are more pronounced, could be informative. But until further work is carried out there is little to conclude regarding the systematic status of the *M. galloprovincialis* from the immunological evidence to date.

Artificial hybridization

There has been only one report to date on artificial hybridization between *M. edulis* and *M. galloprovincialis* (Lubet *et al.*, 1984) Reciprocal crosses have been carried out between *M. edulis* from Luc-sur-Mer, Normandy, France and *M. galloprovincialis* from Vigo, Spain. The control crosses within each mussel type were also accomplished. The F_1 larvae of all crosses were reared under laboratory conditions for a period of one year and then transferred to the wild for two years. During this time all individuals were reared under identical conditions of temperature, salinity, nutrition, population density, etc. While larval mortality was not estimated in the different crosses, other parameters, i.e., incidence of larval abnormalities, time taken to reach metamorphosis, and juvenile (up to 10 mm) and adult (up to 55 mm) mortalities, were recorded. No significant differences were observed between the different crosses. Analysis of the relative growth rates (height/length of shell) for each cross showed no significant difference between 'hybrid' and 'pure' offspring; there was no evidence of heterosis for any of the characters measured. Backcrosses of 'hybrid' F_1 individuals to *M. edulis* F_1 and *M. galloprovincialis* F_1 were also performed and the same techniques were employed in the rearing of the F_2 generations. Unfortunately, all of the F_2 generations, having reached a length of approximately one cm, were lost in a storm. We now know, however, that when *M. galloprovincialis* and *M. edulis* are crossed they can produce fertile 'hybrids' and that these can backcross to the parent forms to produce viable (and perhaps fertile) offspring. Therefore, there is little evidence of genetic incompatibility between the two forms from laboratory-based studies. These results of Lubet *et al.* (1984) support the field evidence, based on morphological and electrophoretic data, of widespread hybridization and introgression between the two forms of mussel in W Europe.

DISCUSSION AND CONCLUSIONS

Morphological, electrophoretic, cytological and immunological studies on the two forms of mussel *M. edulis* and *M. galloprovincialis* indicate that they are very similar to one another with no *single*, morphological or genetic character being clearly diagnostic. This close similarity would not be sufficient grounds, however, for considering the two forms as varieties of a single species since there are several examples in the literature (Gottlieb & Pilz, 1976; Rick *et al.*, 1976; Crawford & Smith, 1982; Harrison & Arnold, 1982) of closely related but distinct species pairs of plants and animals that bear a close morphological and genetic similarity to one another. In almost all such cases, however, there are barriers to gene exchange between the species concerned. For example, in the very closely related cricket species *Gryllus pennsylvanicus* and *G. firmus* (Harrison & Arnold, 1982) laboratory crosses of *G. firmus* males and *G. pennsylvanicus* females produced apparently normal F_1 hybrids and from these F_2 and backcross individuals. The reciprocal cross, however, has consistently failed to produce offspring in the laboratory, indicating that a partial postmating barrier exists between the two species. In the case of the two closely-related plant species *Coreopsis nuecensoides* and *C. nuecensis* (Crawford & Smith, 1982) interspecific hybridization produces vigorous but completely sterile F_1 plants.

Evidence from morphological (Seed, 1978) and electrophoretic (Gosling & Wilkins, 1981; Skibinski & Beardmore, 1979) analyses has indicated that where *M. edulis* and *M. galloprovincialis* are found together they hybridize. (In the *M. edulis/M. galloprovincialis* case the use of the term 'hybridization' is no doubt erroneous (see Mayr, 1963) but is used for the sake of simplicity.) In addition, introgression is widespread especially in parts of Scotland, NE England and at exposed locations on the Atlantic coasts of Ireland. Of course one of the most difficult problems in biology is deciding how much interbreeding can take place before two populations are considered one rather than two species. The evidence so far suggests that the amount of genetic mixing between the two forms "is such as to suggest strongly that there is no isolating barrier between the two types of mussel . . . even in SW England" (where the amount of interbreeding is less); "there is no clear genetic evidence of an absolute reproductive barrier between the types and thus *no good reason to regard them as distinct species* [my italics]" (Skibinski *et al.*, 1983). This conclusion is supported by the results from artificial hybridization studies (Lubet *et al.*, 1984) which indicate that there is little evidence of genetic incompatibility between the two forms. The two types cross readily with each other to produce fertile F_1 individuals and there are no indications of reduced viability or growth rate or increased mortality among the F_1 'hybrids' compared to the parental crosses.

Whether the two forms are in the process of differentiation (incipient sympatric speciation) or whether they are in the process of integrading after a period of allopatry is impossible to determine. The allopatric model is supported by Barsotti & Meluzzi (1968) who believe that *M. galloprovincialis* is a recent derivative of *M. edulis*, having arisen allopatrically in the Mediterranean during a Pleistocene ice age. The present-day distribution of the *M. galloprovincialis* form in W Europe represents a northward expansion of its range since the Pleistocene era. Skibinski *et al.* (1983) suggest that the similarity both in morphological and allozymic characteristics between British and Mediterranean *M. galloprovincialis* are so great as to provide strong support for the allopatry model; there is little supporting evidence for the sympatry model, especially since so few clear examples of sympatric speciation have been described in the literature to date (for a full discussion on this topic see Skibinski *et al.*, 1983). Whether the *M. galloprovincialis* form has arisen in sympatry or in allopatry does not alter the conclusions of this paper, i.e. that the *M. galloprovincialis* form does not merit the rank of full species, but appears, from the evidence to date, to be a variety or ecotype of the larger *Mytilus edulis* species complex.

Accepting that the two mussel types have secondarily come in contact and are freely hybridizing, there are two possible outcomes: either complete intergradation leading to eventual fusion of the two partially differentiated gene pools or, if selection is operating against hybrids, further differentiation and the reinforcement of reproductive isolating barriers. To determine which of these processes is actually occurring would require detailed ecological and genetic analyses of mussel populations of mixed ancestry from selected sites in Britain and Ireland. (For a full discussion on the characteristics and dynamics of hybrid zones the reader is referred to: Mayr, 1963; Lewontin & Birch, 1966; Remington, 1968; Littlejohn *et al.*, 1971; Watson *et al.*, 1971; Watson, 1972; Hunt & Selander, 1973; Moore, 1977; Blackwell & Bull, 1978; Skibinski *et al.*, 1978a, b; Barton, 1979; Gartside *et al.*, 1979; Skibinski & Beardmore, 1979; Woodruff & Gould, 1980; Barton, 1981; Skibinski *et al.* 1983.) Sites such as Rock and Croyde in SW England, where there is little intergradation occurring between the two mussle types, and sites such as exposed locations on the W coast of Ireland, where there is almost complete intergradation, could be surveyed in depth with a view to answering the following questions:

In SW England:

a) Are there barriers to gene exchange between *M. edulis* and *M. galloprovincialis*, i.e. do they have different spawning cyles? Seed (1971) demonstrated differences in the reproductive cycles of the two forms at Rock; here *M. edulis* spawned about eight weeks earlier (May–June) than *M. galloprovincialis* (July–August). It would be interesting to know whether these differences are evident at other sites in the area.
b) Do 'hybrids' have lower fitness than the two parental forms? If chromosomal differences are shown to exist between the two types of mussels in this area then there could be selection against unbalanced heterozygous chromosomal arrangements in the 'hybrids.' Is there selection against introgressed genes? Is there differential introgression of allozyme alleles into the parental gene pools? The use of allozyme markers to measure the extent of introgression between closely related species has been well documented (Hunt & Selander, 1973; Avise & Smith, 1974; Gartside *et al.*, 1979; Skibinski & Beardmore, 1979).
c) Accepting that the two forms of mussels are genetically different, is the extent of differentiation between them increasing or decreasing with time? To answer this question long-term electrophoretic analyses of 'hybrid' populations would be necessary. There may of course be two processes occurring in such populations, as Skibinski *et al.*, 1983 suggest: "the homogenizing effect of interbreeding and the differentiating effect of selection might balance one another resulting in temporal stability of population genetic structure from year to year."

In the case of exposed shore populations on the coasts of Ireland some of the questions might be:

a) Why does the *M. galloprovincialis* form favour the exposed shore environment?
b) Is there evidence of microgeographic separation of the two forms in this habitat, i.e. does the *M. galloprovincialis* form favour the upper shore as opposed to the lower shore?
c) Why is there little or no evidence for the *M. galloprovincialis* form on sheltered Atlantic shores in Ireland, in spite of the long pelagic life (4–6 weeks) of *Mytilus* larvae? Do *M. galloprovincialis* larvae settle but not survive because of competition from *M. edulis* at sheltered sites? This question is probably impossible to answer since the majority of the *M. galloprovincialis* form may die before reaching a size large enough to be sampled for electrophoresis. It must be remembered of course that at some sheltered locations, e.g. Lough Hyne on the

south coast of Ireland (Gosling & Wilkins, 1981) the *M. galloprovincialis* form is abundant and thriving. Why this can occur at some sheltered sites and not at others is not understood.

d) Is the process of intergradation between the two forms on exposed shores (and at other locations, e.g. NE England, parts of Scotland) increasing with time? It is possible that complete fusion of the two gene pools may be in progress and may ultimately produce an ecotype that is particularly well-suited to the rigorous environment of the exposed shore.

In conclusion, the evidence from morphological, electrophoretic, cytological, immunological and artificial hybridization studies now indicate that *M. galloprovincialis* is closely related to *M. edulis* but does not merit the rank of full species. It would appear from the evidence to date that *M. galloprovincialis* is a variety, ecotype or perhaps subspecies of the larger *M. edulis* species complex. The *exact* status of *M. galloprovincialis* still remains an intriguing taxonomic problem, one which will probably occupy the efforts of many investigators in the years to come. In the meantime, it is proposed that the name *Mytilus edulis* var. *galloprovincialis* be used to describe the *galloprovincialis* form of mussel, following Jeffreys (1863).

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